Sep 27, 2019

Ultra-deep, long-read nanopore sequencing of mock microbial community standards

DOI
dx.doi.org/10.17504/protocols.io.x9tfr6n

Josh Quick[1], Sam Nicholls[1], Nick Loman[1], Shuiquan Tang[2]

[1]University of Birmingham; [2]Zymo Research Corporation

Long Read Club
Tech. support email: **n.j.loman@bham.ac.uk**

Josh Quick
University of Birmingham

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.x9tfr6n

External link: https://www.biorxiv.org/content/biorxiv/early/2018/12/04/487033.full.pdf

Protocol Citation: Josh Quick, Sam Nicholls, Nick Loman, Shuiquan Tang 2019. Ultra-deep, long-read nanopore sequencing of mock microbial community standards. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x9tfr6n

Manuscript citation:

Protocol status: Working
We use this protocol and it's working

Created: February 18, 2019

Last Modified: September 27, 2019

Protocol Integer ID: 20499

Keywords: bioinformatics, metagenomics, mock community, nanopore, single-molecule sequencing, real-time sequencing, benchmark, GridION, PromethION, Illumina, de novo assembly

Abstract

Background: Long sequencing reads are information-rich: aiding de novo assembly and reference mapping, and consequently have great potential for the study of microbial communities. However, the best approaches for analysis of long-read metagenomic data are unknown. Additionally, rigorous evaluation of bioinformatics tools is hindered by a lack of long-read data from validated samples with known composition.
Methods: We sequenced two commercially-available mock communities containing ten microbial species (ZymoBIOMICS Microbial Community Standards) with Oxford Nanopore GridION and PromethION. Both communities and the ten individual species isolates were also sequenced with Illumina technology.
Data: We generated 14 and 16 Gbp from GridION flowcells and 150 and 153 Gbp from PromethION flowcells for the evenly-distributed and log-distributed communities respectively. Read length N50 was 5.3 Kbp and 5.2 Kbp for the even
and log community, respectively. Basecalls and corresponding signal data are made available (4.2 TB in total).
Results: Alignment to Illumina-sequenced isolates demonstrated the expected microbial species at anticipated abundances, with the limit of detection for the lowest abundance species below 50 cells (GridION). De novo assembly of metagenomes recovered long contiguous sequences without the need for pre-processing techniques such as binning.
Conclusions: We present ultra-deep, long-read nanopore datasets from a well-defined mock community. These datasets
will be useful for those developing bioinformatics methods for long-read metagenomics and for the validation and comparison of current laboratory and software pipelines.

Guidelines

This protocol has been writen for those wishing to reproduce the DNA extractions used to generate the Nanopore sequencing data presented in the publication.

Materials

MATERIALS

- ZymoBIOMICS Microbial Community Standard **Zymo Research Catalog #**D6300
- ZymoBIOMICS Microbial Community Standard II (Log Distribution) **Zymo Research Catalog #**D6310
- ZymoBIOMICS DNA Miniprep Kit **Zymo Research Catalog #**D4300

STEP MATERIALS

- ZymoBIOMICS Microbial Community Standard II (Log Distribution) **Zymo Research Catalog #**D6310
- ZymoBIOMICS Microbial Community Standard **Zymo Research Catalog #**D6300
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Protocol materials

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Safety warnings

Read the specific MSDS documents accociated with the materials used in the protocol.

Before start

Thaw all frozen reagents on ice, mix well and spin down.

Prepare standard

1 Transfer 75 µL Microbial Community Standard or 375 µL Microbial Community Standard II (Log distribution) to a 1.5 mL Eppendorf tube.

Note

Each 75 ul of the even community is expected to yield 2000 ng and the log community 220 ng therefore a larger starting volume of the later is required.

ZymoBIOMICS Microbial Community Standard II (Log Distribution) **Zymo Research Catalog #**D6310

ZymoBIOMICS Microbial Community Standard **Zymo Research Catalog #**D6300

Retain supernatant

2 Centrifuge the standard at 6,000 xg for 00:05:00 before transferring the supernatant to a new 1.5 mL Eppendorf

Note

The Microbial Community Standard is shipped in DNA/RNA Shield for safety reasons but this causes lysis of Gram-negative species. Retaining the supernatant prevents unecessary shearing during the subsequent bead-beating step.

Resuspend pellet

3 Resuspend the cell pellet in 750 µL Lysis Solution from the ZymoBIOMICS DNA Miniprep Kit and transfer the resuspended cells to a ZR BashingBead Lysis Tube.
ZymoBIOMICS DNA Miniprep Kit **Zymo Research Catalog #**D4300

Bead-beat

4 Load the lysis tube into a FastPrep-24 5G instrument and bead-beat at 6.0 m/s for 2 cycles of 00:00:40 removing the tube and chilling on ice for 00:05:00 between cycles.

Equipment	
FastPrep-24 5G	NAME
Bead beater	TYPE
MP Biomedicals	BRAND
116005500	SKU
https://uk.mpbio.com/fastprep-24-5g-instrument	LINK

5 Centrifuge the lysis tube at 10,000 xg for 00:01:00 and transfer 400 µL supernatant to a Zymo-Spin III-F Filter in a collection tube.

6 Centrifuge the filter column at 8,000 xg for 00:01:00 and transfer 400 µL filtrate to a new 15 mL Falcon tube.

7 For the Microbial Community Standard add 45 µL of the supernatant from Step 2 and 1.485 mL Binding buffer to the tube and mix well. For the Microbial Community Standard II (Log distribution) add 225 µL of the supernatant from Step 2 and 2.025 mL instead.

8 Load 800 µL onto a Zymo-Spin IIC-Z Column, centrifuge at 8,000 xg for 00:01:00 and discard flow through. Repeat as many times as needed to process all the mixture.

9 Wash and elute the sample as per the ZymoBIOMICS DNA Miniprep Kit instruction manual.

10 Prepare sequencing libraries using the Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies) using 1400 ng input DNA and loading 50 ng (MinION) or 400 ng (PromethION) completed library onto the flowcell.